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Effects of Ethylene Glycol Monomethyl Ether on Various Parameters of Testicular Function in the F344 Rat

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These studies were designed to define the target cell for ethylene glycol monomethyl ether (EGME) action in the rodent testis, and to compare fluid production and protein secretory patterns in the testes of treated and control animals. Adult F344 rats were treated with 150 mg/kg/day, 5 day/week, and sacrificed 1, 2, 4, 7 or 10 days after the first dose. In the testes of treated animals, the most sensitive cells were the premeiotic and meiotic spermatocytes. As length of treatment continued, the younger transitional spermatocytes, and more pachytene spermatocytes, were affected. Early and late stage spermatids did not appear affected, nor was the visual appearance of spermatogonia changed by exposure to EGME. Fluid production, androgen-binding protein secretion and electrophoreograms of proteins found in the ligated rete testis were also unchanged by EGME, showing that these indices of Sertoli cell function were not affected by EGME. The histologic and biochemical data suggest that both early and late spermatocytes are targets for EGME in the testis, and that the Sertoli cells are relatively unaffected.

Introduction

The National Toxicology Program is currently undertaking an initiative in male reproductive toxicology. The selected approach to understanding gonadal toxicity involves first identifying the cells which are the initial targets of chemical action in the testis or epididymis, and subsequently examining possible mechanisms of action for the compounds in question. Simultaneously, the program is dedicated to identifying potential early indicators of testicular damage which could be used for screening purposes. Ethylene glycol monomethyl ether (EGME) was one of the first compounds to be analyzed in this manner, and part of that work is presented here.

Experimental

Histologic Studies

After preliminary dose-setting studies, adult F344 rats ($n = 8$) were treated PO with EGME in distilled water, 150 mg/kg/day, 5 day/week; controls ($n = 4$) received daily doses of distilled water. Animals were killed by exsanguination under deep methoxyfluorane anesthesia and perfused with Karnovsky's fixative on days 1, 2, 4, 7 and 10 after the start of dosing. Tissues were embedded in glycol methacrylate, and stained with PAS to allow staging of spermatogenesis by the method of Leblond and Clermont (*1*). Other animals were treated identically and were used for biochemical studies; these will be described later.

Mild histologic effects were seen 24 hr after the first dose, when some meiotic spermatocytes lost the monochromatic appearance of their cytoplasm, and were seen to undergo nuclear condensation (Fig. 1). Occasional late pachytene spermatocytes (premeiotic cells) and some younger, transitional spermatocytes were also seen to be affected. These latter effects became more pronounced on the second day; zygotene and pachytene spermatocytes, and more transitional spermatocytes, were seen to be necrotic. By days 4 and 7, these effects were advanced, with the result that the populations of young, round spermatids were decimated in the affected tubules as their precursor spermatocytes were killed. This resulted in tubules containing Sertoli cells, spermatogonia, some early spermatocytes, and the usual number of elongated spermatids, but largely depleted of late-stage spermatocytes and their division products, the early stage spermatids (Fig. 2). Tubular stages VI–X were not visibly affected, and spermiation (the release of condensed spermatids) was apparently normal. At days 7 and 10, numerous multinucleated giant cells were evident, containing mostly spermatocyte nuclei (Fig. 3); some sloughed spermatids were present in the tubular luminae. Not surprisingly, the epididymis

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contained numerous shed spermatocytes and some nonnucleated, PAS-positive inclusions (Fig. 4); as duration of exposure increased, the numbers of these increased and they appeared further away from the testis.

In summary, the changes visible at the light microscopic level appear to be specific for spermatocytes. The meiotic cells seem slightly more sensitive, although premeiotic pachytene cells and the younger transitional (or first stage pachytene) spermatocytes are also targets for EGME actions. This effect on spermatocytes produced a maturation depletion effect on the spermatid population, and yielded tubules which were devoid of early stage spermatids.

Biochemical Studies

There is some recent data which buttresses the long-held belief that the Sertoli cells support and nurture the germ cells during their maturation to condensed spermatids (*2–4*). The effect on spermatocytes, although apparently due to a direct effect on those cells, could be secondary to an effect of EGME on the Sertoli cells. To study the possible effects of EGME on Sertoli cells, and as part of the broader effort to characterize testicular toxicants, some animals (n = 6/group) were treated with EGME as above. Approximately 16 hr before sacrifice, the animals were anesthetized and a small ventral midline incision was made. Each testis was externalized, the efferent ducts were ligated, and the organs replaced. The following day, the animals were sacrificed by carbon dioxide asphyxiation, and the rete testis was punctured with the broken end of a glass capillary tube. The resulting fluid (30–50 μL) was collected, centrifuged to removed the cells and assayed for total protein by dye binding assay (Bio-Rad) and for androgen binding protein (ABP) by steady-state electrophoresis (*5*). An aliquot was subjected to SDS-polyacrylamide gel electrophoresis on 1.5-mm thick slab gels by using an acrylamide gradient of 5 to 20%. In addition, six treated and six control animals were sacrificed without prior ligation.

Total protein levels in control groups varied between 2.2 ± 1.01 and 3.27 ± 0.99 mg/mL (mean ± SD). Protein levels in the treated groups rose insignificantly over the course of treatment, from 2.87 ± 0.61 to 3.91 ± 1.40 mg/mL; there was no significant difference between treated and control groups at any time.

Testicular weight as a fraction of body weight is indicated in Table 1. It can be seen that the testis

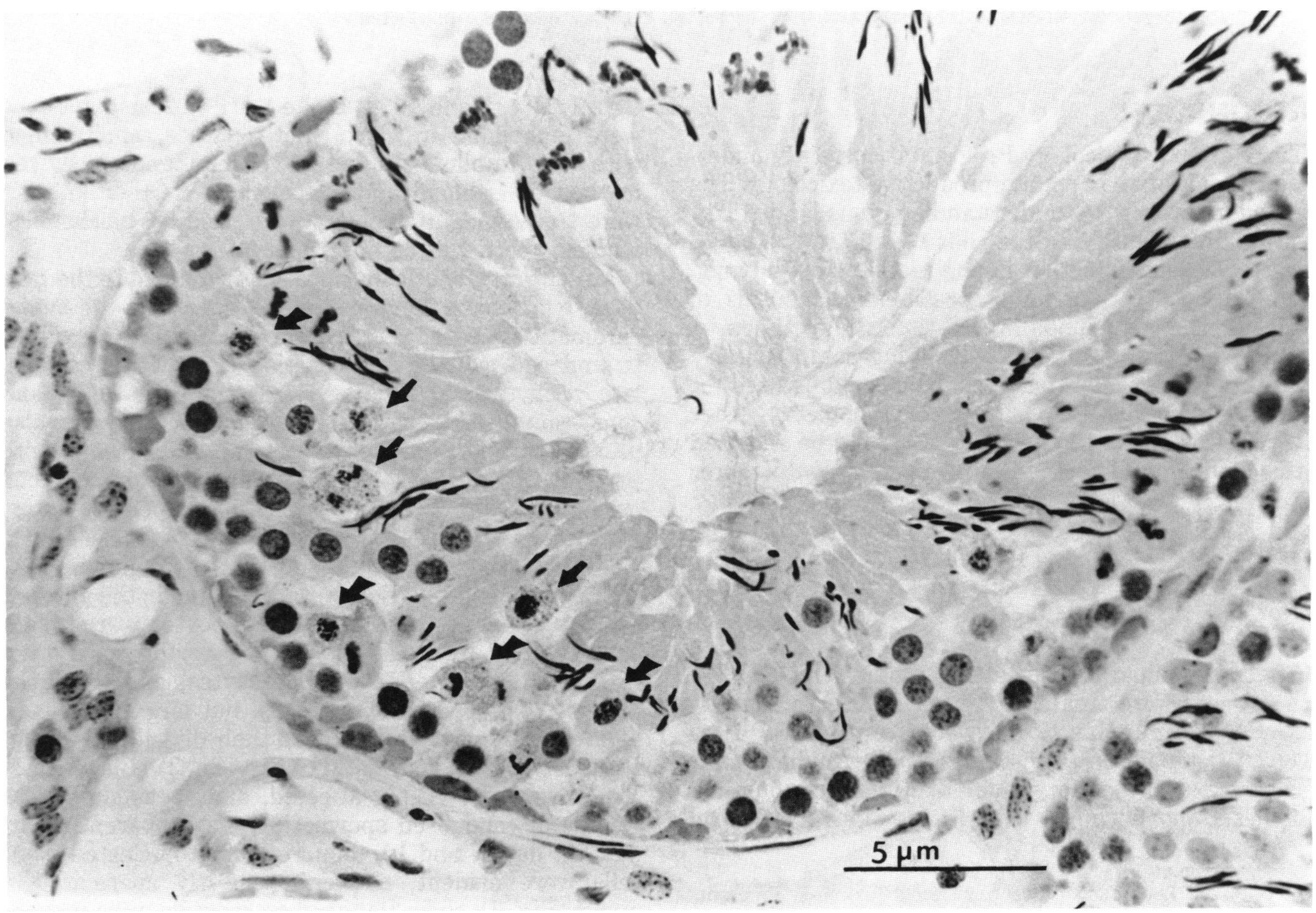

FIGURE 1. Stage XIV tubule from F344 rat 24 hr after a single dose of 150 mg EGME/kg. Note necrotic spermatocytes (arrows). Scale bar is 5 μm.

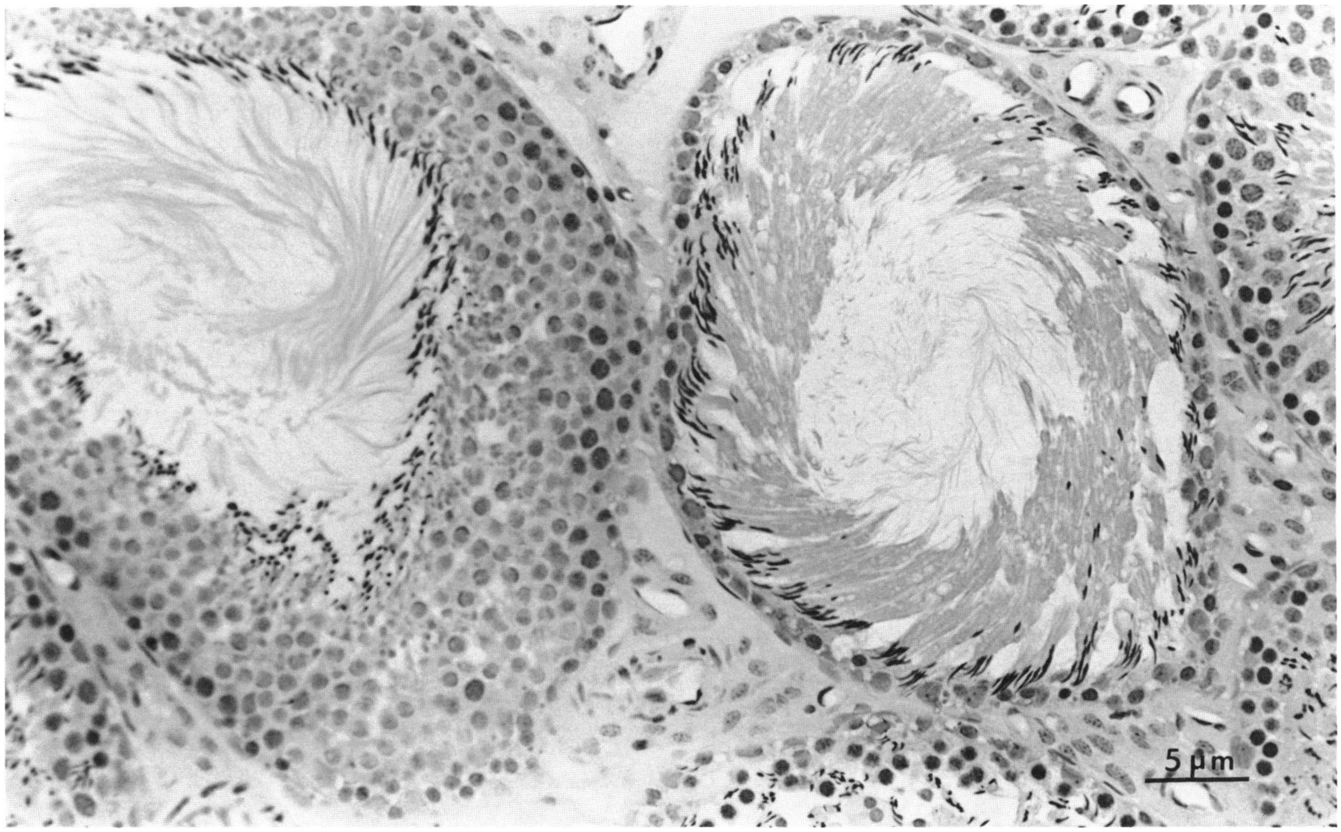

FIGURE 2. Seminiferous tubules from F344 rat 4 days after the start of dosing with 150 mg/kg/day. Tubule on the right is depleted of spermatocytes and early spermatids. Scale bar is 5 µm.

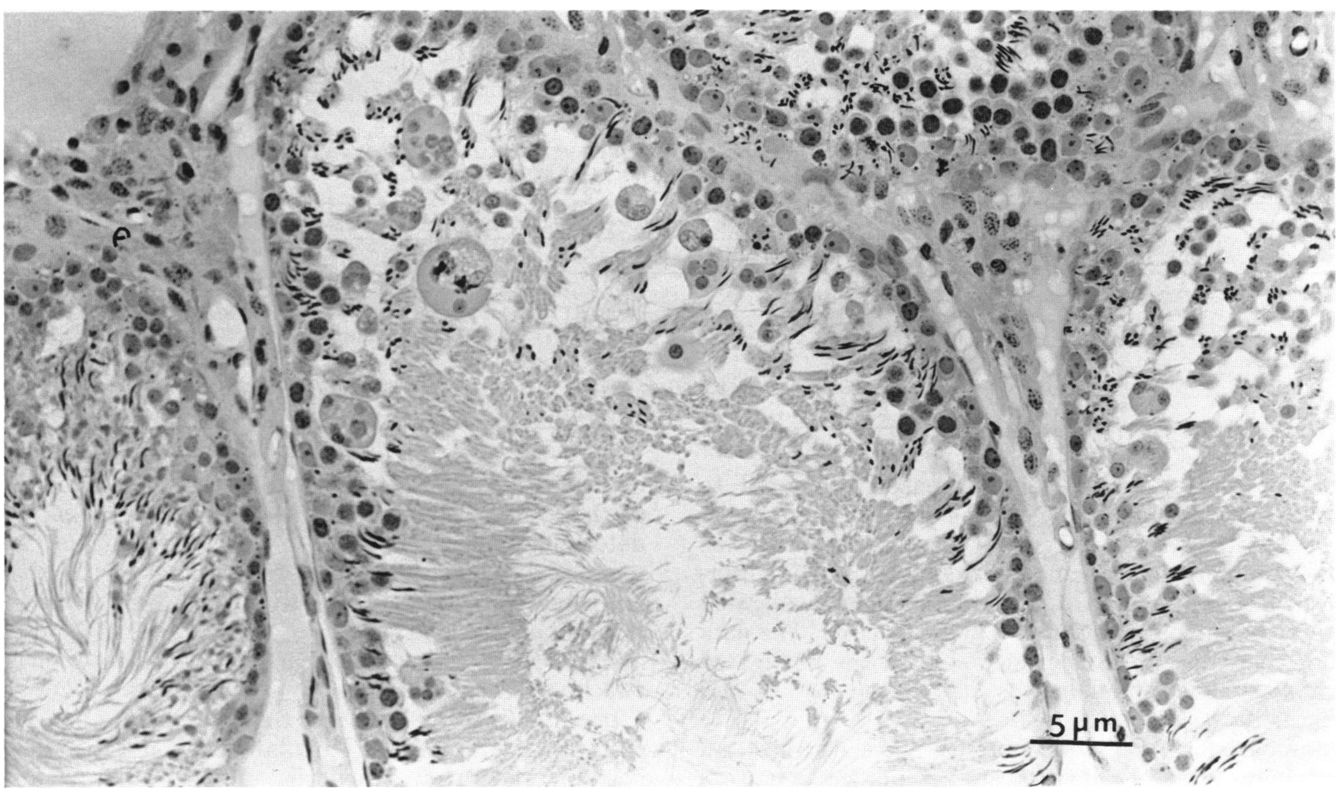

FIGURE 3. Seminiferous tubules from F344 rat 7 days after the start of dosing with 150 mg/kg/day, 5 day/week, showing presence of multinucleated giant cells. Scale bar is 5 µm.

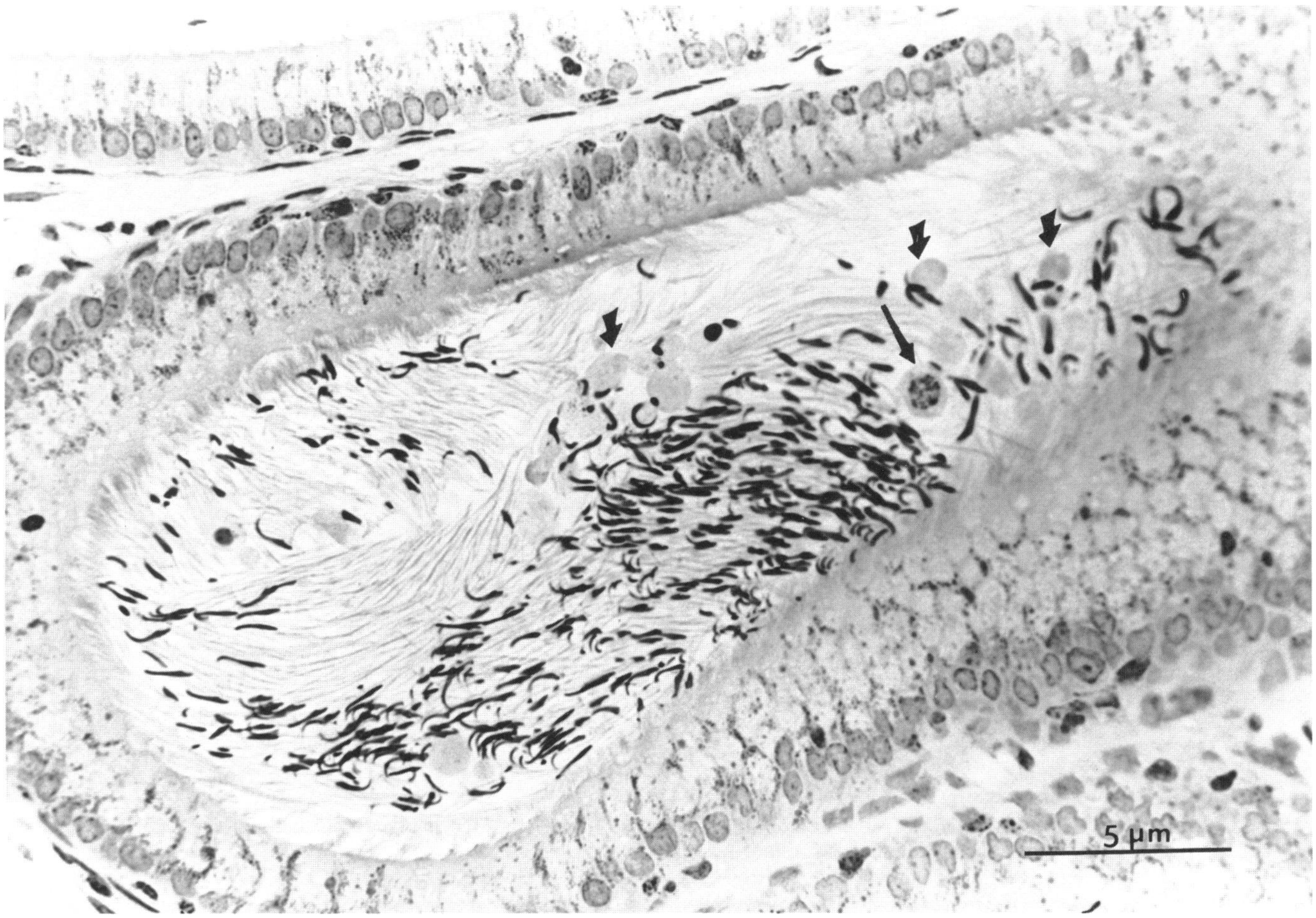

FIGURE 4. Tubule in caput epididymis of F344 rat 4 days after start of dosing with 150 mg/kg/day. Note PAS-positive bodies (short arrows) and spermatocyte (long arrow). Scale bar is 5 µm.

Table 1. Testis weight/body weight calculations from ligated and nonligated rats. Treatment groups with the same superscript were significantly different, $p < 0.05$.

	Testis weight/body weight (g/g), means ± SD (n = 6)			
Treatment	Day 2	Day 4	Day 7	Day 10
Ligated				
EGME	0.0135 ± 0.0004 ^{a}	0.0126 ± 0.0003 ^{c}	0.0123 ± 0.0009 ^{f,g}	0.0111 ± 0.0005 ^{j,k}
Control	0.0139 ± 0.0005 ^{b}	0.0125 ± 0.0007 ^{e}	0.0140 ± 0.0006 ^{f,h}	0.0155 ± 0.0009 ^{j,l}
Nonligated				
EGME	0.0113 ± 0.0005 ^{a}	0.0091 ± 0.0004 ^{c,d}	0.0101 ± 0.0015 ^{g,i}	0.0082 ± 0.0004 ^{k,m}
Control	0.0119 ± 0.0004 ^{b}	0.0101 ± 0.0002 ^{e,d}	0.0117 ± 0.0004 ^{h,i}	0.0111 ± 0.0014 ^{l,m}

weights of treated animals decreased compared to controls, presumably due to the loss of germ cells. However, the weight increase in the ligated testes was similar in both treated and control animals. The difference between ligated testis weight and nonligated weight gives an indication of fluid production. The data show that, despite a drop in weight in the nonligated testis, fluid production was unchanged by EGME treatment.

Samples for ABP determination were run in a gel containing tritiated dihydrotestosterone, the gels were sliced, and the radioactivity in each slice was determined. A plot of cpm versus slice produced a peak at the location of ABP. The area under this peak was determined on a planimeter (Zeiss Videoplan) and expressed in square millimeters. Samples from control and treated animals from the same day were run simultaneously. Data from ABP assays are shown in Table 2. There was no significant difference between the two groups at any time.

In addition to ABP, the Sertoli cells secrete up to 60 proteins (*6*) into both the tubular lumen and the blood. It would seem that a majority of the protein components in rete testis fluid are secreted by the semi-

Table 2. Area under the peak, androgen-binding protein.

Treatment	Area under the peak, androgen-binding protein (mean ± SD)			
	Day 2	Day 4	Day 7	Day 10
Control	876.6 ± 120.0 (n = 6)	766.1 ± 87.9 (n = 5)	807.0 ± 72.0 (n = 6)	808.7 ± 70.3 (n = 6)
EGME	1023.8 ± 125.3 (n = 4)	688.1 ± 94.0 (n = 5)	973.3 ± 192.4 (n = 5)	965.5 ± 177.0 (n = 5)

niferous tubules (*7*). Electrophoresis of rete testis fluid has shown that treating animals with EGME as described above does not consistently alter the protein patterns seen in this fluid; samples from treated animals did not show the presence of new bands, nor did they lose established bands.

Discussion

Ethylene glycol monomethyl ether is notable as a testicular toxicant, partly because the testis is affected at doses lower than those which affect other organs, and partly to the specificity of the effect in the testis. The initial changes are seen in spermatocytes and as one changes the dose, one can vary the generations of spermatocytes affected (*8,9*). Preliminary analysis of other data from our laboratory suggests an effect on either spermatogonia or Sertoli cells at slightly higher doses than those used in this study (Chapin et al., in preparation). From the data presented above, it would seem that late stage spermatocytes are the most sensitive to this dose of EGME, while the younger, transitional spermatocytes in earlier stage tubules are slightly less sensitive.

Spermatocytes have been reported to be the targets for at least one other compound. Patanelli and Nelson (*10*) reported that both early and late stage spermatocytes in stage VIII tubules appeared affected by treatment with diethyldicarbamylmethyl-2,4-dinitropyrrole. They also reported that with increasing time after the single dose of dinitropyrrole, tubules in earlier stages began to exhibit necrotic changes. This suggests that some cells were affected by the compound, but did not begin to undergo necrosis until they had matured somewhat, even though they began cell death before they reached the age of the cells showing the initial effects.

We observed similar phenomena in the EGME-treated animals, although the initial target cells differ from those in the dinitropyrrole-treated rats. It is interesting, although currently unexplainable, that both early and late stage spermatocytes were affected by both compounds. This could be due to toxic effects on the cells themselves, or might be mediated through an interruption of Sertoli cell function.

Many of the proteins secreted by the Sertoli cells are absorbed by germ cells and other Sertoli cells before reaching the rete testis (*7*); thus, fluid from the rete testis under free flow conditions is not a reflection of the fluid in the seminiferous tubule. For example, protein levels fall from approximately 6 mg/mL in the tubules, to approximately 1 mg/mL in rete testis fluid (*7*). In addition, Koskimies and Kormano (*11*) demonstrated that of the 20 bands visible in electrophoreograms of seminiferous tubule fluid, three were not visible in fluid from the rete testis. It is possible that the secretion of one or more of the resorbed proteins was changed by EGME treatment; using the methods we employed, we would not see such a change. However, the fact that neither ABP levels, nor the profile of proteins detectable in the rete testis fluid, nor fluid production, was changed in the treated animals indicated that at least these indices of Sertoli cell function were unaffected by EGME. Whether the secretion of smaller molecules or lipids was affected by EGME is unknown.

In summary, spermatocytes were seen to be the first cells to undergo necrotic changes after treatment of adult F344 rats with ethylene glycol monomethyl ether. Analysis of several indices of Sertoli cell function were examined and found to be unaffected by EGME treatment.

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